Process Relationships for Evaluating the Role of Light-induced Inactivation of Coliphages at Selected Beaches and Nearby Tributaries of the Great Lakes

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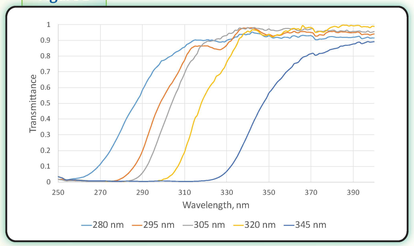
Introduction

Past studies have indicated that sunlight plays an important role in altering densities of coliphages, other indicator microorganisms and pathogens in aquatic environments. Fate and transport modeling of bacteriophage requires mathematical relationships that describe the wavelength dependence for photoinactivation (biological weighting functions (BWFs), also called action spectra). In this study we define BWFs as the photoinactivation rate constants normalized to the irradiance at various wavelengths. Photoinactivation of bacteriophage is defined by loss of culturability (ability to form plaques). The objective of this presentation is to provide action spectra for photoinactivation of four F-specific/somatic coliphages and then to illustrate the use of the action spectra in modeling the photoinactivation of the phages at selected Great Lakes beaches. (Fig. 1)

Experimental

MS2 (an fRNA bacteriophage) and Northeastern Ohio fRNA Coliphages (both RNA based) along with θX-174 and Northeastern Ohio Somatic Coliphages (DNA based) were plated for enumeration (EPA Method 1602) after irradiation by solar simulator using the double layer agar method. The Ohio samples were isolated from wastewater samples collected from a Cleveland, Ohio treatment plant. The photoinactivation experiments were conducted by irradiating the phage suspensions in a Solar Light LS-1000 solar simulator equipped with a 1 kW xenon-arc lamp and various UV-cutoff filters that modified UV exposure during a series of experiments (see Figure 2 for UV transmission of the filters). The spectral irradiance of the filtered light was measured using an Optronics OL 756 spectroradiometer. Samples of each phage were prepared in phosphate buffered saline (pH 7.0) at concentrations of about 10^7 PFU/mL and 30 mL of the phage suspension were added to 18 mm OD quartz tubes for each set of irradiations. The quartz tubes were kept at 20°C by immersion in a thermostated water bath during the 48-hour irradiations. We conducted kinetic runs in triplicate with each phage and showed that photoinactivation could be described by first order kinetic expressions with up to seven orders of magnitude decreases in enumerated phage densities (Figure 3). Thus, photoinactivation data were represented by first order rate constants computed for each data set.

Figure 2



BWFs were computed using the irradiance data and first order rate constants observed with each of the five cutoff filters in place. We found that satisfactory fits to produce the BWFs could be developed using exponential equations of the general form:

$$BWF(\lambda) = EXP(a * \lambda + b)$$

Figure 3

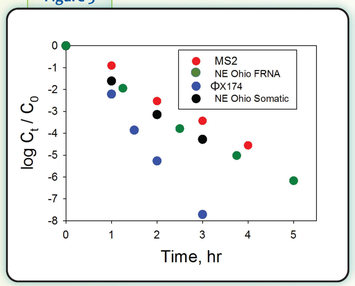
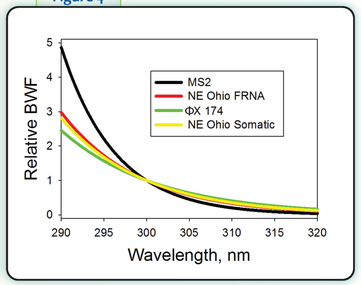


Figure 4



Where λ is the wavelength in nanometers and *a* and b are fitting parameters. We used Excel software to solve for the values of the parameters *a* and b as described originally by Rundel (1). To facilitate comparison of the spectral shape of the BWFs, we normalized their values to unity at 300 nm (Figure 4).

Results & Discussion

Action spectra, or plots of BWFs versus wavelength, provide useful inputs to models that predict photoinactivation in the aquatic environment. We are using a model developed by Madronich at the National Center for Atmospheric Research (NCAR) called TUV to estimate the irradiance for various times and places. The photoinactivation rate constant k_i in a water body can be modeled by integrating the cross product of the downwelling irradiance E_d and the BWF over the photoactive wavelengths of sunlight (which are usually in the UV).

$$k_i = \int E_d(z,\lambda)(BWF(\lambda))\, d\lambda$$

In Figure 5 the BWF for the somatic phage and irradiance for mid-July at Washington Park Beach in on Lake Michigan near Michigan City, IN are compared. The BWF increases significantly with shorter wavelengths in the 280-315 nm region (called UV-B) whereas the irradiance decreases sharply with shorter wavelength in the UV-B. The product of the irradiance and BWF is referred to as the weighted irradiance for photoinactivation (Figure 5). For all the phages the integration of the weighted irradiance over all solar wavelengths is greatest in the UV-B (Figure 6) and it increases in the order MS2, Ohio FRNA, Ohio somatic, and θX-174 somatic phages. Also the somatic phages have somewhat greater weighted irradiances at longer wavelengths. These results indicate that phage photoinactivation should be quite sensitive to environmental factors that influence underwater UV-B radiation. In particular changes in total ozone in the atmosphere and elevation can modulate UV-B reaching the water surface. The effects of ozone changes and elevation are included in the NCAR model. Other factors such as clouds and aerosols are known determinants of UV-B entering the water. We have coupled TUV with data bases maintained by NOAA and USGS to automatically input data on total ozone and elevation for various GPS coordinates.

As part of our modeling efforts we have also examined the depth dependence of phage inactivation. Results of these modeling studies indicate that there are significant differences in the depth dependence of the four phages that were examined in this work (Figure 7). We estimated the depth dependence of underwater UV irradiance using the following:

$$E_d(z,\lambda) = E_d(0,\lambda)\, e^{-K_d(\lambda)*z}$$

Where $E_d(z,\lambda)$ and $E_d(0,\lambda)$ are irradiances at depth z and at the water surface, respectively. The water attenuation coefficients $K_d(\lambda)$ were estimated using data obtained from water samples collected at Great Lakes beach sites. The results shown in Figure 7 were computed for the Washington Park Beach. Other modeling results show that the phages are strongly protected from photoinactivation at all the tributary sites that we examined. The colored organic matter in the water is primarily responsible for this UV protection.

Other recent studies of direct phage photoinactivation are cited in the references.

Figure 5

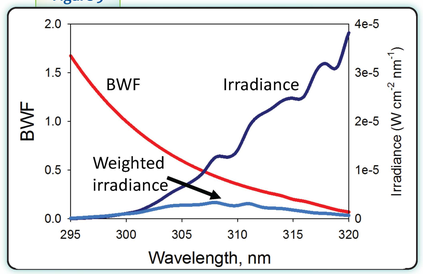


Figure 1

Rundel Approach for Evaluating Photoinactivation of Microorganisms in Aquatic Environment

1. Determine biological weighting functions (BWF), e.g. Rundel technique (Rundel, Physiol. Plant., 58, (1986) 360-366.)
2. Use measured or estimated spectral diffuse attenuation coefficients to quantify light attenuation at beach sites.
3. Use BWF, light attenuation coefficients and solar irradiance to model depth dependence of photoinactivation at different times and locations.

Figure 6

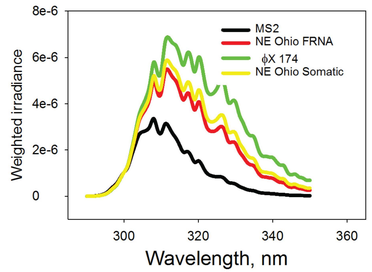
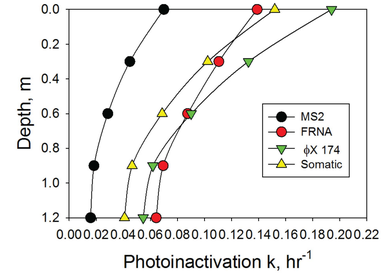


Figure 7



Conclusions

(1) Our results show that photoinactivation kinetics of four bacteriophages (two fRNA and two somatic coliphages) can be described by first order rate expression up to six orders of magnitude change in concentration. Rates and half-lives are approximately independent of phage concentration.

(2) Biological weighting functions (BWFs) (action spectra) determined for sunlight-inactivation of these phages show that the UV-B part of sunlight (280-315 nm) drives their photoinactivation in the aquatic environment.

(3) Models developed using the BWFs and environmental data as inputs were used to simulate phage inactivation in aquatic environments. Results show that the near-surface inactivation rate constants are sensitive to atmospheric parameters such as total ozone, cloud cover, and aquatic parameters such as absorption coefficients of the water column.

(4) Modeled results for Great Lakes sites indicate that photoinactivation is strongly phage dependent with somatic phages more photoactive than fRNA phages; MS2, an fRNA bacteriophage, was the least photoreactive. Simulations of the depth dependence in meter-deep water at beach sites show that UV-attenuating substances in the water retard photoinactivation by factors of 2-3 compared to the surface rates.

References

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